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Comparative analysis of amino acid profiles from the 13 mtDNA protein-coding genes in New Zealand White and Indonesia local rabbits

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ABSTRACT

This study aimed to characterize the amino acid profiles of 13 mtDNA protein-coding genes in Indonesian local rabbits and New Zealand White (NZW) rabbits. Genomic DNA (gDNA) was extracted from liver tissue to obtain complete mtDNA sequences using long-read sequencing technology (Nanopore). The mtDNA sequences were aligned to identify amino acid variations in the 13 mtDNA protein-coding genes, including ND1, ND2, ND3, ND4, ND4L, ND5, ND6, COX1, COX2, COX3, ATP6, ATP8, and CYTB. Results showed that the amino acid composition in both strains was largely conserved, with leucine as the dominant residue. Minor variations were observed, such as higher phenylalanine in Indonesia local rabbits and slightly lower isoleucine in NZW rabbits, suggesting potential differences in energy metabolism and physiological adaptation. Low proportions of cysteine, arginine, and aspartate were found in both strains, reflecting their limited structural role. Overall, the amino acid composition patterns of mtDNA were highly conserved (dominated by Leu, Ile, Phe, and Ser), although small but significant differences in certain genes, such as ND4L, COX1, and ND2, may be associated with genetic adaptation, domestication history, and physiological performance of the two strains.

Introduction

Mitochondrial DNA (mtDNA) is a small, circular genetic molecule that plays a central role in cellular energy metabolism through genes encoding components of the oxidative respiratory chain (Taanman, 1999). In mammals, including rabbits (Oryctolagus cuniculus), mtDNA contains 13 protein-coding genes (PCGs) that are essential for oxidative phosphorylation. Because mtDNA is maternally inherited, relatively free from recombination, and has a higher mutation rate compared to the nuclear genome, mtDNA analysis is widely used in phylogenetic studies, population genetics, metabolic adaptation, and investigations of genetic relationships among populations or breeds.

The amino acid profile constituting the proteins encoded by mtDNA reflects codon-level changes that may affect the structure and function of mitochondrial proteins (da Fonseca *et al*., 2008). Nonsynonymous mutations that alter amino acid residues can influence the efficiency of the electron transport chain, energy production, and sensitivity to oxidative stress, ultimately impacting the physiological performance of animals for example, growth, disease resistance, temperature tolerance, and feed conversion. Therefore, mapping and comparing the amino acid profiles of mtDNA protein-coding genes across rabbit populations or breeds can provide valuable insights into local adaptation, mitochondrial functional variation, and the potential development of genetic markers for breeding programs.

The commercial New Zealand White (NZW) and Indonesia local rabbits represent two groups that are often compared in the context of production and adaptation. Local rabbits are thought to have undergone natural selection and adaptation to tropical environmental conditions in Indonesia (climate, local feed resources, and endemic diseases) (Setiaji *et al*., 2023), whereas NZW, although widely farmed globally including in Indonesia, originated from breeding lines developed in temperate regions. These differences may be reflected in distinct mtDNA mutation patterns and, consequently, in the amino acid profiles of mitochondrial proteins information that is relevant for understanding mechanisms of energy adaptation, resilience to environmental stress, and production performance.

Recently, many mitochondrial genome studies in livestock and model animals have highlighted the presence of mtDNA variants associated with physiological or adaptive traits (Yu *et al*., 2009; Xiao *et al*., 2018; Brajkovic *et al*., 2023; Brajkovic *et al*., 2025). However, comprehensive studies comparing the amino acid profiles of the entire set of mtDNA protein-coding genes (PCGs) between Indonesia local rabbits and NZW raised in Indonesia remain scarce or undocumented. This knowledge gap limits our understanding of whether local adaptation or breeding history influences mitochondrial function at the amino acid level, as well as the potential application of such variants as selection markers or breeding criteria. Based on these considerations, the present study aimed to characterize the amino acid profiles of the 13 mtDNA protein-coding genes in populations of Indonesia local rabbits and NZW raised in Indonesia.

Materials and methods

Ethical Approval

All experimental protocols were reviewed and approved by the Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (Approval No. 59–01/A-01/KEP-FPP)

Tissue collection and DNA extraction

This study utilized genomic DNA (gDNA) extracted from the liver tissue of an Indonesia local rabbit and New Zealand White (NZW) rabbit (Oryctolagus cuniculus). Rabbits were slaughtered and the liver tissue was harvested. Approximately 10 g of tissue was collected and preserved in Falcon tubes containing ethanol. Genomic DNA was then isolated from

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these tissue samples following the manufacturer's protocol using the Quick-DNA MagBead Plus Kit (Zymo Research, USA). The extracted gDNA was assessed for quality and quantity, after which mitochondrial DNA (mtDNA) was enriched using the REPLI-g Mitochondrial DNA Kit (Qiagen, Germany). The enriched mtDNA was subsequently used for library preparation employing the enhanced mtDNA protocol.

MtDNA sequencing analysis

Mitochondrial DNA (mtDNA) was sequenced using long-read sequencing technology by the Oxford Nanopore Technologies GridION platform (Zascavage *et al*., 2019a; Zascavage *et al*., 2019b). Sequencing runs were managed with MinKNOW (v21.11.17), and base calling was performed in high-accuracy mode using Guppy (v5.1.13) (Wick *et al*., 2019). Read quality was visualized with NanoPlot (v1.40.0) (De Coster *et al*., 2018), and reads were aligned to the Oryctolagus cuniculus mitochondrial reference genome (GenBank AJ001588) using minimap2 (v2.24). Assembly of filtered reads was performed with Flye (v2.8.3), followed by polishing four times with Racon (v1.5.0) and three times with Medaka (v1.5.0) (Vaser *et al*., 2017). The final sequences were annotated and visualized using MitoZ (v2.4) (Meng *et al*., 2019), and sequence quality was assessed with Quast (v5.0.2) (Gurevich *et al*., 2013).

Data analysis

Data analysis was performed using MEGA11 software (Tamura *et al*., 2021). The complete mitochondrial DNA (mtDNA) sequences of Indonesian local rabbits and New Zealand White rabbits were aligned to identify nucleotide and amino acid variations. Comparative analyses were carried out to examine differences in amino acid composition across the 13 mtDNA-encoded proteins that consisting of ND1, ND2, ND3, ND4, ND4L, ND5, ND6, COX1, COX2, COX3, ATP6, ATP8 and CYTB.

Results

The distribution of amino acid composition encoded by protein-coding genes (PCGs) in the mitochondrial genome of Indonesia local rabbits is presented in Figure 1, while the distribution in New Zealand White rabbits is illustrated in Figure 2. These figures provide a clear overview of the relative proportions of each amino acid constituting the mitochon-

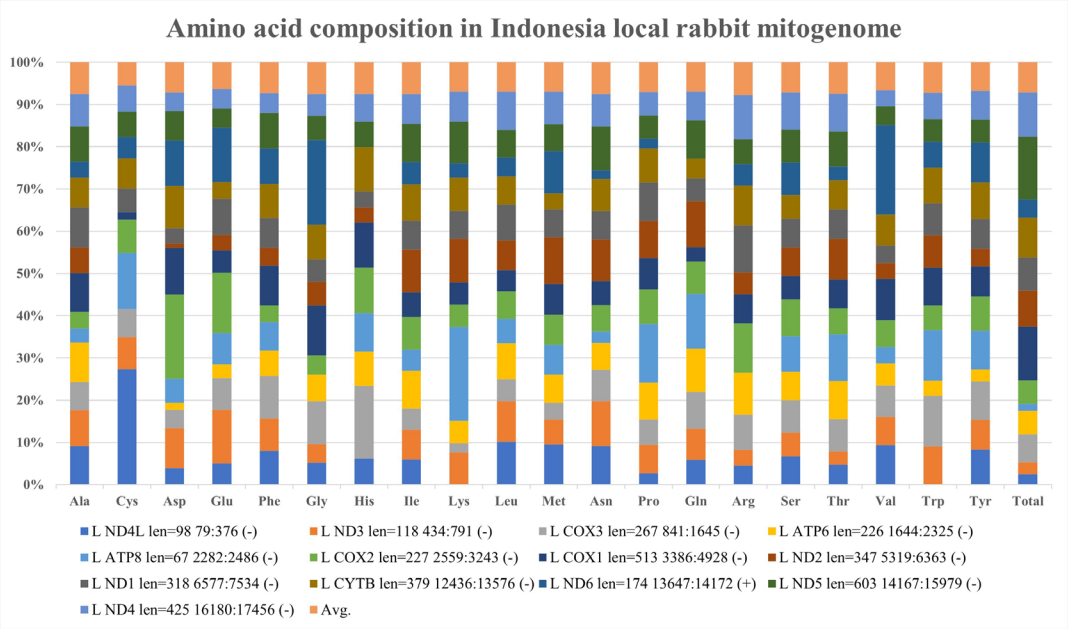

Figure 1. Amino acid composition in Indonesia local rabbit mitogenome.

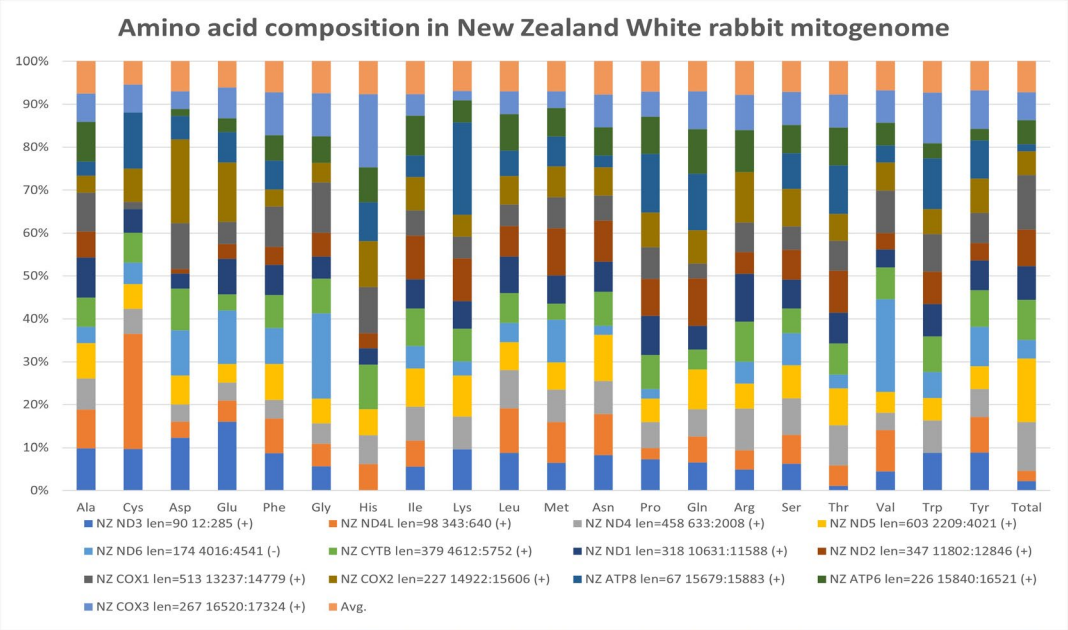

Figure 2. Amino acid composition in New Zealand White rabbit mitogenome.

drial proteins in both rabbit strains. Overall, the comparative visualization shows a similar pattern, with several dominant amino acids. Nevertheless, although the general distribution pattern remains conserved, differences in the proportion of certain amino acids can be observed, indicating genetic variation and physiological adaptation between the two strains. Presenting the data in the form of illustrations not only facilitates the identification of dominant and minor amino acids but also provides a foundation for deeper comparative analysis of each amino acid's contribution to cellular respiration and energy metabolism in both Indonesia local and New Zealand White rabbits. Thus, the visualization in these figures serves as an important interpretative tool to understand differences in mitochondrial genome composition and their implications for the biological performance of each strain.

Discussion

The analysis of amino acid composition encoded by protein-coding genes (PCGs) in the mitochondrial genome of Indonesia local rabbits and NZW rabbits revealed a relatively uniform distribution pattern, albeit with certain variations in the proportions of specific amino acids. These findings provide important insights into both the genetic conservation and differences between strains, which may have implications for energy metabolism and physiological adaptation. In general, leucine (Leu) was the most dominant amino acid in both strains. In NZW rabbits, the average proportion of Leu reached 15.8%, while in Indonesia local rabbits it approached a similar value, indicating that Leu plays a crucial role in mitochondrial protein formation. The dominance of Leu is a common feature in animal mitogenomes, considering that codons for Leu are more abundant than those of other amino acids (Gibson *et al*., 2005; Jia and Higgs *et al*., 2008). In addition to Leu, amino acids with high frequencies included isoleucine (Ile), threonine (Thr), serine (Ser), alanine (Ala), and phenylalanine (Phe). The relatively high percentages of these amino acids suggest that mitochondrial proteins in both rabbit strains require residues with nonpolar as well as polar properties to maintain structural integrity and enzymatic function in cellular respiration.

Conversely, cysteine (Cys) consistently exhibited the lowest proportion (<1%) in both Indonesia Local and NZW rabbits. This is a common feature in mitogenomes, as Cys is relatively rarely utilized in mitochondrial proteins (Moosmann and Behl, 2008). In addition to Cys, arginine (Arg) and aspartate (Asp) were also found in very low proportions, each accounting for less than 2% of the total amino acid composition in both rabbit strains. The low abundance of Cys can be explained by its nature as a semi-essential amino acid that occurs in limited amounts in mitochondrial proteins, although it still plays an important role in disulfide bond formation and protein structural stability (Habich *et al*., 2019). Meanwhile, Arg, which is involved in the urea cycle and nitric oxide synthesis, was also detected in small amounts. Its limited proportion may reflect its more prominent role in specific metabolic pathways rather than in the assembly of major structural proteins. On the other hand, Asp plays a vital role as a precursor in the citric acid cycle and in the biosynthesis of various non-essential amino acids (Reitzer, 2004). The low proportion of Asp in mtDNA PCGs may indicate that its contribution is more focused on energy metabolism pathways rather than on the construction of structural protein components.

Several genes exhibited more pronounced variation in amino acid composition between the two strains. This may reflect differences in codon usage that could potentially influence the stability or efficiency of mitochondrial enzyme complexes (Jia and Higgs, 2008). Phenylalanine (Phe) was relatively higher in Indonesia local rabbits compared to NZW rabbits. Phe is an aromatic amino acid that plays an important role in protein synthesis and serves as a precursor for various bioactive compounds; thus, its differential abundance may indicate metabolic variation between the two strains. The higher Phe content in Indonesia local rabbits might be associated with physiological adaptations to tropical environments, which demand greater efficiency in protein metabolism. Another notable variation was observed in isoleucine (Ile), with NZW rabbits showing slightly lower proportions. Ile is an essential amino acid that plays a key role in energy metabolism through the catabolism of branched-chain amino acids (Adeva-Andany *et al*., 2017). This difference may suggest a variation in the capacity of the two strains to utilize energy substrates in muscle tissues.

Although the differences in amino acid composition among certain mtDNA PCGs are not extreme, they remain biologically relevant. Such variations may reflect physiological adaptations, for instance in energy metabolism between Indonesia local rabbits and NZW rabbits, the latter being selectively bred for meat production with a faster growth rate. The history of domestication, in which selective breeding of NZW rabbits for productivity may have introduced subtle shifts in codon preference and amino acid usage, could explain part of this divergence. On the other hand, the high degree of evolutionary conservation, as indicated by the overall similarity in distribution patterns, highlights the essential and highly preserved role of mitochondrial proteins across strains. These findings are significant not only for understanding the molecular basis of rabbit physiology but also for practical applications: in breeding programs, where strains with more efficient energy metabolism may be prioritized; in animal health research, particularly in relation to differential resilience against metabolic or environmental stress; and in phylogenetic studies, where variation in amino acid composition can serve as an additional marker to complement genetic analyses in delineating relationships across strains or species.

Conclusion

Although the overall amino acid composition pattern of mitochondrial PCGs in Indonesia local and NZW rabbits is highly conserved (dominated by Leu, Ile, Phe, and Ser), small but significant differences are observed in certain genes such as ND4L, COX1, and ND2. These variations may be associated with differences in genetic adaptation, domestication history, and the physiological performance of the two strains.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this paper.

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